Video Article

DNA Electroporation, Isolation and Imaging of Myofibers

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URL: http://www.jove.com/video/53551
DOI: doi:10.3791/53551

Keywords: Molecular Biology, Issue 106, Muscle, myofiber, sarcolemma, DNA electroporation, fluorescence, confocal microscopy

Date Published: 12/23/2015

Citation: Demonbreun, A.R., McNally, E.M. DNA Electroporation, Isolation and Imaging of Myofibers. *J. Vis. Exp.* (106), e53551, doi:10.3791/53551 (2015).

Abstract

Mature muscle has a unique structure that is amenable to live cell imaging. Herein, we describe the experimental protocol for expressing fluorescently labeled proteins in the flexor digitorum brevis (FDB) muscle. Conditions have been optimized to provide a large number of high quality myofibers expressing the electroporated plasmid while minimizing muscle damage. The method employs fluorescent tags on various proteins. Combining this expression method with high resolution confocal microscopy permits live cell imaging, including imaging after laser-induced damage. Fluorescent dyes combined with imaging of fluorescently-tagged proteins provides information regarding the basic structure of muscle and its response to stimuli.

Video Link

The video component of this article can be found at http://www.jove.com/video/53551/

Introduction

Individual myofibers are large, highly organized syncytial cells. There are a few cell-culture models for muscle; however, these models have as their major limitation that they do not fully differentiate into mature myofibers. For example, the C2C12 and L6 cell lines are derived from mice and rats, respectively [1-4]. Under conditions of serum starvation, the mononuclear "myoblast-like" cells cease proliferation, undergo cell cycle withdrawal, and enter into the myogenic program forming multinucleated cells with sarcomeres, referred to as myotubes. With prolonged culture conditions, myotubes may exhibit contractile properties and "twitch" in culture. Human cell lines have also now been established [5]. In addition to these immortalized cell lines, mononuclear myoblasts can be isolated from muscle and under the similar conditions of serum starvation will form myotubes. These cell lines and primary myoblast cultures are highly useful because they can be transfected with plasmids or transduced with viruses and used to study basic cell biological processes. However, these cells, even when induced to form myotubes, lack many of the salient features of mature muscle organization. Specifically, myotubes are much smaller than individual mature myofibers and lack the normal shape of myofibers. Critically, myotubes lack transverse (T-) tubules, the membranous network required for efficient Ca^{2+} release throughout the myoplasm.

An alternative method to primary myoblast or myogenic cell lines entails using mature myofibers. Transgenesis can be employed to establish expression of tagged proteins, but this method is costly and time consuming. *In vivo* electroporation of mouse muscle has emerged as a preferred method for its speed and reliability [6-10].

Methods for *in vivo* electroporation and the efficient isolation of myofibers have been optimized for the mouse flexor digitorum brevis (FDB) muscle [6]. The methods can be completed readily and are minimally invasive to induce *in vivo* expression from plasmids. This approach is now combined with high resolution imaging methods including imaging after laser disruption of the sarcolemma [6,7]. The combination of fluorescent dyes and expression of fluorescently labeled proteins can be used to monitor cell biological processes in mature myofibers.

Protocol

The methods in this study were performed in ethical accordance with the Northwestern University Feinberg School of Medicine Institutional Animal Care and Use Committee (IACUC) approved guidelines. All efforts were made to minimize suffering.

1. Experimental Procedure for *in vivo* Electroporation of the Flexor Digitorum Brevis (FDB) Muscle Bundle in Mouse

1. Design plasmids for *in vivo* expression using a promoter known to express in mammalian cells (*e.g.,* cytomegalovirus, CMV) or in muscle (*e.g.,* Muscle Creatine Kinase, MCK) [6,7]. Larger plasmids may express at lower levels. The CMV promoter has been used extensively [6,7].